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Original Article

Dentistry Section

Screening the Drug Resistance Property Among Aerobic Pathogenic Microorganisms of Dental Caries in North-Western Indian Population: A Preliminary Study

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ABSTRACT

Aims and Objectives: To evaluate the emerging drug resistance among the caries pathogens isolated from carious dentine microbiologically.

Materials and Methods: Specimens from dental caries were collected from 75 patients referred to Department of Conservative Dentistry and Endodontics, Vyas Dental College. Microbiological processing of all the samples was done within three hours to isolate the caries pathogens. The samples were inoculated on agar medium (Nutrient agar, Mac-conkey's agar) at 37^{0}C for 48 hours aerobically. The identification of strains was done by observing colony morphology and gram's staining. The predominant isolates were subjected to antimicrobial sensitivity test (Kirby Bauer's method). Statistical analysis of the isolates was done using paired t-test.

Results: Out of 75 patients more common isolates were *Staphylococcus aureus, Klebsiella, Pseudomonas aeruginosa, Yeast.* The predominant were *Staphylococcus* and *Pseudomonas* species. Newer antibiotics were proved to be effective against these predominant strains after evaluating antibiotic sensitivity tests.

Conclusion: Although *Streptococcus mutans (S.mutans)* is the most prevalent microorganism seen in dental caries, the role of other microorganisms like *Staphylococcus, Pseudomonas* in initiation and progression of caries is evident from this study. Further extensive and large scale studies need to be conducted for better understanding the role of these microorganisms in dental caries.

Keywords: Antibiotic resistance, Caries pathogens, Gram stain, Kirby bauer method, *Pseudomonas aeruginosa, Staphylococcus* species, *Streptococcus* species

INTRODUCTION

Dental caries is one of the most prevalent diseases of the oral cavity, having a multifactorial etiology and characterized by the destruction of organic and inorganic matrix. It has no sex predilection and equally effects individuals from all age groups [1]. In 17th century, Leeuwenhoek found the role of bacteria in the etiology of dental caries and coined the term "little animalcules" [2]. Later in 1890, the association of these bacteria with pulpal and periapical pathologies was reported by WD Miller. There has been greater research on bacteria causing caries initiation and early caries progression, although microbiology of dentinal caries is yet to be fully characterized [3].

The most commonly isolated microorganism from a carious lesion is *S.mutans* and has been target microorganism for routine cultures. However, Clarke in 1924 noted that caries could develop in the absence of *S.mutans* and a wide variety of microorganisms (*Lactobacillus acidophilus*, *Staphylococcus*, proteolytic bacteria, anaerobic organism including *Prevotella*, *Vellionella*, *Fusobacterium, Actinomyces viscosus,* etc*)* were identified which aid in caries initation and progression [4,5].

Multiple factors, such as the interaction of bacteria, diet, oral hygiene, plaque, immunity, time and host respons, all influence dental caries initiation and progression. It is uniformly agreed that caries cannot occur without microorganisms. Bacterial accumulation is mediated by both bacterially derived polymers and salivary components. Although saliva plays an important role in optimal oral health and new research suggests that salivary pH is even more critical to the caries development and progression, as low salivary pH promotes the growth of acidogenic bacteria in the oral cavity [6].

From past so many years, surgical treatment has been followed in many dental offices to remove the decayed tooth structure. The flaw in this approach is that the causative organisms are not removed completely and to overcome these problems antimicrobials like penicillin, erythromycin was used. But with time, resistance has developed among the various caries pathogens against these antimicrobial drugs. This results in compelling evidence to progress with clinical assessments of antibiotic susceptibility and various microbiological efforts to understand the limits of this everlasting problem [7].

AIM

The aim of study was to evaluate the caries pathogens from dentinal caries other than *S. mutans* and to screen the emergence of drug resistance among the caries causing pathogenic microorganisms.

MATERIALS AND METHODS

The study was conducted from May 2014 to September 2014 in the Department of Oral Pathology at Vyas Dental College and Hospital. In the present study 75 patients with permanent dentition, having dental caries were selected. The presence of caries was confirmed by visual and radiographic examination. Soft carious dentin, easily removable with spoon excavator was isolated from the lesion and was used as specimen for culturing. The collected specimens were processed after obtaining the informed consent from the patients

and a proforma was recorded for each study case to analyse the age, sex, occupation, marital status, food habits and detailed clinical examination.

After the removal of superficial plaque and debris overlying the lesion, the carious zone of decalcified and partially decalcified dentine was washed with sterile saline, carious dentine was then excavated with sterile spoon excavators which was transferred in to vials of sterile normal saline to a concentration of approximately 10 mg (wet weight) of dentine per ml prior to processing [Table/Fig-1].

The collected samples were brought to the microbiology laboratory and were processed within 3 hours of collection. Samples were dispersed in the transport medium by using a Vortex mixer and the broth was incubated at 37°C for 2 hours. Later on broth was inoculated on to, nutrient agar and mac-conkey agar which was incubated at 37°C for 48 hours each.

The isolates were then identified by colony morphology; grams staining and were stored at 4°C until subjected for the antimicrobial susceptibility test. Briefly, colonies of the test organisms were touched with a sterile loop and transferred into sterile Mueller Hinton broth under aseptic conditions and were incubated at 37°C for 2 hours until the density of each microbial suspension reached to 10^6 colony forming units per ml (CFU/ ml).

One hundred microliter of the inoculum of each test organism was spread as lawn cultures onto sterile Mueller Hinton agar plates using L-rods to achieve a confluent growth. Discs of first, second, third line of antibiotics, along with newer (beta lactam) antibiotics were placed on the culture plates of *staphylococcus* and *pseudomonas* strains [Table/Fig-2], the plates were then incubated at 37°C for 18 hours and the zone of inhibition was measured by using Kirby Bauer's disc diffusion method [8].

RESULTS

In this study out of 75 patients more common isolates were *Staphylococcus aureus, Klebsiella, Pseudomonas aeruginosa, Yeast*. The predominant were *Staphylococcus* and *Pseudomonas* [Table/Fig-3].

The most prevalent micro-organisms were *Staphylococcus aureus* and *Pseudomonas aeroguinosa*. These strains were isolated and the antibiotic sensitivity tests were carried out. Microorganisms which were sensitive showed zones of inhibition, which were resistant, showed absence of zone of inhibition [Table/Fig-4]. After which, statistical analysis of the samples was done using paired t-test and p-value was calculated. P-value was found to be significant in case of first, second and third line antibiotics against both strains (*Pseudomonas* and *Staphylococcus*), whereas it was highly significant in case of newer antibiotics [Table/Fig-5]. Thereby, newer antibiotics were proved to be effective against the microbial pathogens.

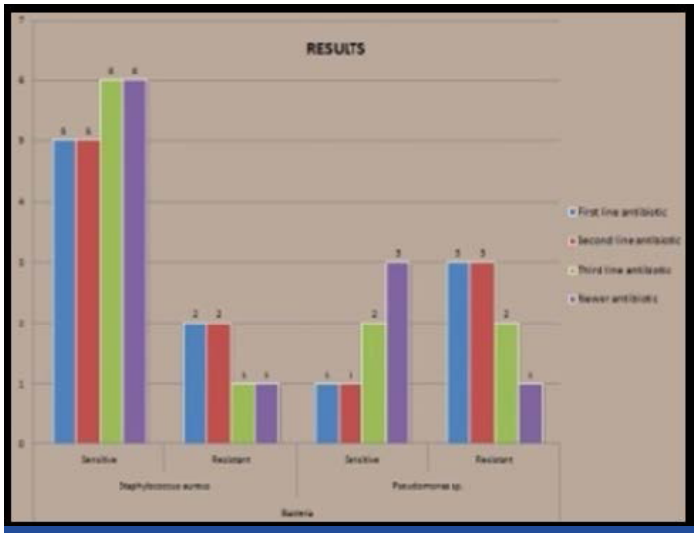
[Table/Fig-1]: 10mg of carious dentin in 1ml of normal saline

Generations of antibiotics	Names of Antibiotics used
First line of antibiotics	Pencillin (500mg), tetracycline (500mg), ciprofloxacin (500mg), ofloxacin (500mg)
Second line of antibiotics	Cefixime (500mg), Levofloxacin (500mg) Vancomycin (500mg), azithromycin (500mg)
Third line of antibiotics	Cefepime (1000 mg), Meropenem (500mg) Tobramycin (1200mg)
Newer antibiotics (beta lactam)	Cefazolin (500mg), Amoxyclav (625mg) Carbenicillin (764mg)

[Table/Fig-2]: Various generations of antibiotics used

Bacteria	Gender		Total
	Male	Female	
Staphylococcus aureus	24	10	34
Pseudomonas sp.	19	5	24
Klebsiella	1	3	4
Yeasts	1	4	5
No Microrganism	5	3	8
Total	50	25	75

[Table/Fig-3]: Bacterial distribution according to gender



[Table/Fig-4]: Graph showing antibiotic efficacy against *staphylococcus* and *pseudomonas* strains

Staphylococcus aureus	Results		
	t- test	df	p <.05
First line antibiotics	16.316	6	.001
Second line Antibiotics	16.316	6	.001
Third line Antibiotics	17.728	6	.001
Newer antibiotics	17.728	6	.0005
Pseudomonas aeruginosa	Results		
	t- test	Df	p <.05
First line antibiotics	16.734	3	.006
Second line Antibiotics	12.969	3	.006
Third line Antibiotics	12.170	3	.014
Newer antibiotics	12.170	3	.015

[Table/Fig-5]: Chart showing p-value calculated in both bacterial strains

DISCUSSION

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, which often leads to cavitation in the teeth. It is a complex and dynamic process where a multiple factors influence and initiate the progression of disease [9]. The microbiology of caries is complex and dependent upon interactions of protective and pathologic factors in saliva and plaque biofilm as well as balance between the cariogenic and non-cariogenic microbial population

that reside in saliva [10,11]. Some of the oral cariogenic bacteria like *Staphylococcus aures, Klebisella, Pseudomonas aeruginosa* were also identified in the present study.

The mutans group of *streptococci* play a central role in the initiation of caries on the smooth surfaces and fissures of the crowns in the teeth of adults and children. They also have a potent etiologic role in the induction of root surface caries [12]. Although *S. mutans* is most commonly found microorganism in dental caries, but the presence of *staphylococci* has been recognized in few detailed studies [13].

It is evident from this study that the dentinal caries were contaminated with cultivable microorganisms other than *streptococci*, which is in full agreement with the results of studies done by several authors. Alicia et al., identified *staphylococcus* species and *Candida ablicans* in dental caries and periodontal pockets of periodontal disease patients [14]. Oluremi et al., found prevalence of *staphylococcus*, followed by other microorganisms (*S.mutans, Lactobacilli*) in dental caries [15]. Daniyan et al., found prevalence and susceptibility of *staphylococcus* isolates of dental caries in a secondary health care institution [16]. Similarly the present study was aimed to detect the role of microflora other than *S. mutans* in caries as not much research has been done on the other cultivable microorganisms present in dental caries. *Staphylococci was* the predominant strain, which was in conjunction with previous studies. The exact role of *staphylococcus* in caries is still not clear, but it is believed to play an important role in caries initiation and progression.

Dental caries has been treated by removal of diseased tooth structure or faulty restorations prior to placement of materials to restore form and function. The underlying thought for this approach must be that surgical removal of the nidus of infection will inhibit the disease processes. However, the flaw in this method is that the removal of the demineralized/diseased tooth structure does not result in complete elimination of the causative infection because if foci of infection persist it will result in recurrence, so the next step is to treat the remaining infection with effective antimicrobial agents [3]. It has been reported that the introduction of antimicrobials in the prophylactic treatment has reduced the infection, but the long term use of these antibiotics could be compromised by emergence of resistant strains [3].

The utilization of antimicrobials in treating dental caries has gained much attention worldwide. But the widespread concern is emerging drug resistance among the pathogenic population has worsened the treatment regimens in dentistry. The developing resistance of bacteria to these common antimicrobial agents is a cause of concern for the dentist [3]. The resistance developed to antimicrobial agents can arise from the selection of resistant strains among naturally susceptible species or from the ingress of newer strains of naturally resistant species. Greater use of a single antimicrobial agent in a given environment directly dictates the rate of resistance among the different microbial populations [17].

A potential post antibiotic era is threatening present and future of medical advances in treating dentinal caries [18]. Overuse and abuse of antibiotics are the main factors responsible for the development of resistance against antibiotics. Bacteria such as *Staphylococcus aureus*, *S. mutans*, *Pseudomonas aeruginosa, Klebsilla* are few among the antibiotic resistant bacteria [19].

Amongst all these caries pathogens, development of resistance to antimicrobial agents by *Staphylococci* is a major concern primarily because they are still frequently associated with hospital and community – acquired infections. These organisms exhibit remarkable versatility in their behaviour towards antibiotics, with some strains becoming resistant to commonly used drugs like pencillin, erythromycin, etc [20]. Therefore, in context to lowering down the antimicrobial resistance rate; it is imperative to consider routine susceptibility testing. Such tests could be useful in improving the efficacy of treatment.

In this study, three generation of antibiotics, along with newer antibiotics were used to assess the changing pattern of resistance to antibiotics in bacteria isolated from carious lesions. Antibiotics used in the present study have shown to be effective against *Staphylococcus* and *Pseudomonas*, which was in conjunction with previous studies. The rationale of using different spectrum of antibiotics was to determine their efficacy against these microorganisms and also to assess the resistant strains developed against the known antibiotics. Both broad spectrum antibiotics (tetracycline, amoxyclav) and narrow spectrum antibiotics (ciprofloxacin, cefazolin, carbenicillin, vancomycin etc) were used in the present study, which were proven to effective against the bacteria to be tested.

Staphylococcus were found to be most predominant out of which 20% were found to be resistant to penicillin and 1% were found to be resistant to erythromycin. These values were significantly less when compared to a previous studies done by Uwaezuoke et al., and Dwivedi et al., in which they found 95.8% and 48% samples resistant to penicillin respectively [17,21]. Second most predominant bacteria isolated was *pseudomonas*, most of its samples were found to be resistant to fluoroquinolones, which is in conjunction with a previous study done by Mac Dougall et al., [20]. When comparing the various generations of antibiotics 80% of the *staphylococci* were sensitive to all lines of antibiotics and only 10 % were found resistant to antibiotics in present study. 70% of *pseudomonas* was found to be resistant to first three lines of antibiotics although 73% samples exhibited sensitivity to the newer antibiotics.

Selection of an antibiotic regimen should always be based on knowledge of the efficacy of an antibiotic for the bacteria. It should also be remembered that dental caries represent an ecosystem of bacteria in which by-product of one species of bacteria may be nutrient of other bacteria. Thus, an antibiotic that is effective against a few species of bacteria in polymicrobial infection, may indirectly affect all or some of these bacteria in that ecosystem. Therefore, the study results depict that the growing resistance among pathogenic bacteria against the commonly prescribed antibiotics will be eliminated by the usage of newer antibiotics [22].

Recently, newer advances like hard tissue lasers, chemomechanical methods etc. have been introduced for conservatively removing carious lesions while simultaneously nanotechnology in dentistry has been developed as a drug delivery system for control of biofilm dependent oral diseases. Such advances will be helpful in successful management of these diseases which will certainly open a new avenue to overcome diseases like dental caries [23-25]. Dental caries, a disease of great antiquity, is not a disease of past since it is reappearing in many countries as a public health crisis. It is therefore, important that public health be caught on prevention, early recognition and reporting to dental offices for proper prognosis. This underscores the need of early diagnosis, to rule out the antimicrobial resistance against the microorganisms for proper management and increasing the efficacy of treatment.

CONCLUSION

There are different ways of accomplishing the removal of the pathogens by developing targeted antimicrobials and is part of the continuing evolution of the treatment of oral infection that produces the clinical manifestations of dental caries. As a profession, we are slowly moving away from the purely surgical approach to treating this disease. Regardless of which of these or other strategies emerges as a winner in the war on caries, it is most interesting that these technologies will serve whole other areas of health care as well.

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